

Supplementary Material

Tmprss2 specific miRNAs as promising regulators for SARS-CoV-2 entry checkpoint.

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Supplemental Method 1

The approach for the miRNA Q-PCR employed in the present study is based on miScript II RT kit and miScript SYBR® Green PCR Kit (Qiagen, Madrid, Spain), <https://www.qiagen.com/us/products/discovery-and-translational-research/pcr-qpcr-dpcr/qpcr-assays-and-instruments/mirna-qpcr-assay-and-panels/miscript-ii-rt-kit/#orderinginformation>.

Within this context, reverse transcription reactions performed using miScript HiSpec Buffer ensuring the selective conversion of mature miRNAs and the targets of miScript PCR Controls into cDNA. Mature miRNAs are polyadenylated by poly (A) polymerase and reverse transcribed into cDNA using oligo-dT primers (polyadenylation and reverse transcription are performed in parallel in the same tube). The oligo-dT primers have a 3' degenerate anchor and a universal tag sequence on the 5' end, allowing amplification of mature miRNA in the real-time PCR step. The combination of polyadenylation and the universal tag addition ensures that miScript Primer Assays do not detect genomic DNA. The primers used to perform QPCR with the miRNA-derived cDNA are the universal reverse primer provided in the kit miScript SYBR® Green PCR Kit and a unique forward primer that allows specific amplification of the target of interest. The universal reverse primer anneals to the cDNA universal sequence tag that was added to the 5' end of all cDNA species by the RT adaptor primer during 1st-strand cDNA synthesis (Forero et al., 2019). For the expression analysis, U6 was used as housekeeping gene with relative miRNA expression calculated in comparison to miRNA control (miC) and mock control (MC) (Kaur et al., 2020).

Supplementary Tables

Supplementary Table 1 Primer Sequences

Gene	Forward	Reverse
Tmprss2	ACTCTCTTCCTGCTGGGTCA	AGCAGCCACCAATAAACCCAC
Hsa-miR214-3p	ACAGCAGGCACAGACAGGCAGT	-----
Hsa-miR-98-5p	TGAGGTAGTAAGTTGTATTGTT	-----
Hsa-miR32-3p	CAATTTAGTGTGTGTGATATTT	-----

Tmprss2 Transmembrane Serine Protease 2

Supplementary Table 2 Probability confidence score of individual nucleotides in the 3'UTR Tmprss2 for target accessibility.

Site ID	Nucleotide position in the input sequence	Nucleotide	Probability that this nucleotide is unpaired
miR214			
1	5	A	0.964
1	6	U	0.994
1	7	U	0.982
1	8	G	0.946
1	9	A	0.938
1	10	G	0.918
1	11	A	0.990
1	12	U	0.947
1	13	C	0.932
1	14	U	0.981
1	15	U	0.957

1	16	C	0.950
1	17	C	0.956
1	18	U	0.948
1	19	G	0.965
1	20	C	0.984
1	21	U	0.973
1	22	G	0.903
miR98			
1	1	U	0.723
1	2	G	0.678
1	3	U	0.737
1	4	U	0.999
1	5	U	1.000
1	6	C	0.974
2	7	U	0.999
2	8	A	0.839
2	9	C	0.808
2	10	A	0.746
2	11	C	0.901
2	12	A	0.905
2	13	U	1.000
2	14	U	0.996
2	15	G	0.964
2	16	C	0.994
2	17	U	0.999
2	18	A	0.971
2	19	C	0.973
2	20	C	1.000
2	21	U	1.000
2	22	C	0.996
2	23	A	0.996
miR32			

1	2	G	0.924
1	3	A	0.964
1	4	A	0.977
1	5	U	0.979
1	6	A	0.984
1	7	U	0.964
1	8	C	0.922
1	9	A	0.909
1	10	U	0.134
1	11	G	0.073
1	12	C	0.075
1	13	A	0.192
1	14	A	0.923
1	15	A	0.594
1	16	U	0.986
1	17	A	0.993
1	18	A	1.000
1	19	A	0.999
1	20	U	0.945
1	21	U	0.566

Supplementary Table 3 Target accessibility and probability profile for human Tmprss2 (3'UTR) – anti-oligo binding.

S.No.	miRNA	Target	Antisense oligo	GC content (%)	oligo binding energy (kcal mol ⁻¹)	Average unpaired probability for target site nucleotides	binding site disruption energy (kcal mol ⁻¹)
1	hsa-miR-98-5p	UUCUACACAUUGCUACCUCA	TGAGGTAGCAATGTGTAGAA	40.0	-18.6	0.953	-0.3
2	hsa-miR-214	CCCCAUUGAGAUCUCCUGC	GCAGGAAGATCTCAATGGGG	55.0	-22.0	0.965	-0.4
3	hsa-miR-32	UGCUGGAUGACUUGAGAUGA	TCATCTCAAGTCATCCAGCA	45.0	-9.5	0.595	5.8
4	hsa-let-7a-5p	GUUUCUACACAUUGCUACCU	AGGTAGCAATGTGTAGAAAC	40.0	-8.7	0.645	4.2
5	hsa-let-7b-5p	GCUGGAUGACUUGAGAUGAA	TTCATCTCAAGTCATCCAGC	45.0	-9.5	0.610	5.7
6	hsa-let-7c-5p	GCUGGAUGACUUGAGAUGAA	TTCATCTCAAGTCATCCAGC	45.0	-9.6	0.624	5.5
7	hsa-let-7d-5p	CUCCUAAUAAAGACAUACCC	GGGTATGTCTTTATTAGGAG	40.0	-11.1	0.675	6.9
8	hsa-let-7e-5p	GCUGGAUGACUUGAGAUGAA	TTCATCTCAAGTCATCCAGC	40.0	-9.6	0.596	5.8
9	hsa-let-7f-5p	GCUGGAUGACUUGAGAUGAA	TTCATCTCAAGTCATCCAGC	45.0	-9.6	0.608	5.7
10	hsa-let-7g-5p	UGCUGGAUGACUUGAGAUGA	TCATCTCAAGTCATCCAGCA	45.0	45.0	0.600	5.9
11	hsa-let-7i-5p	UGCUGGAUGACUUGAGAUGA	TCATCTCAAGTCATCCAGCA	45.0	-9.6	0.593	5.8

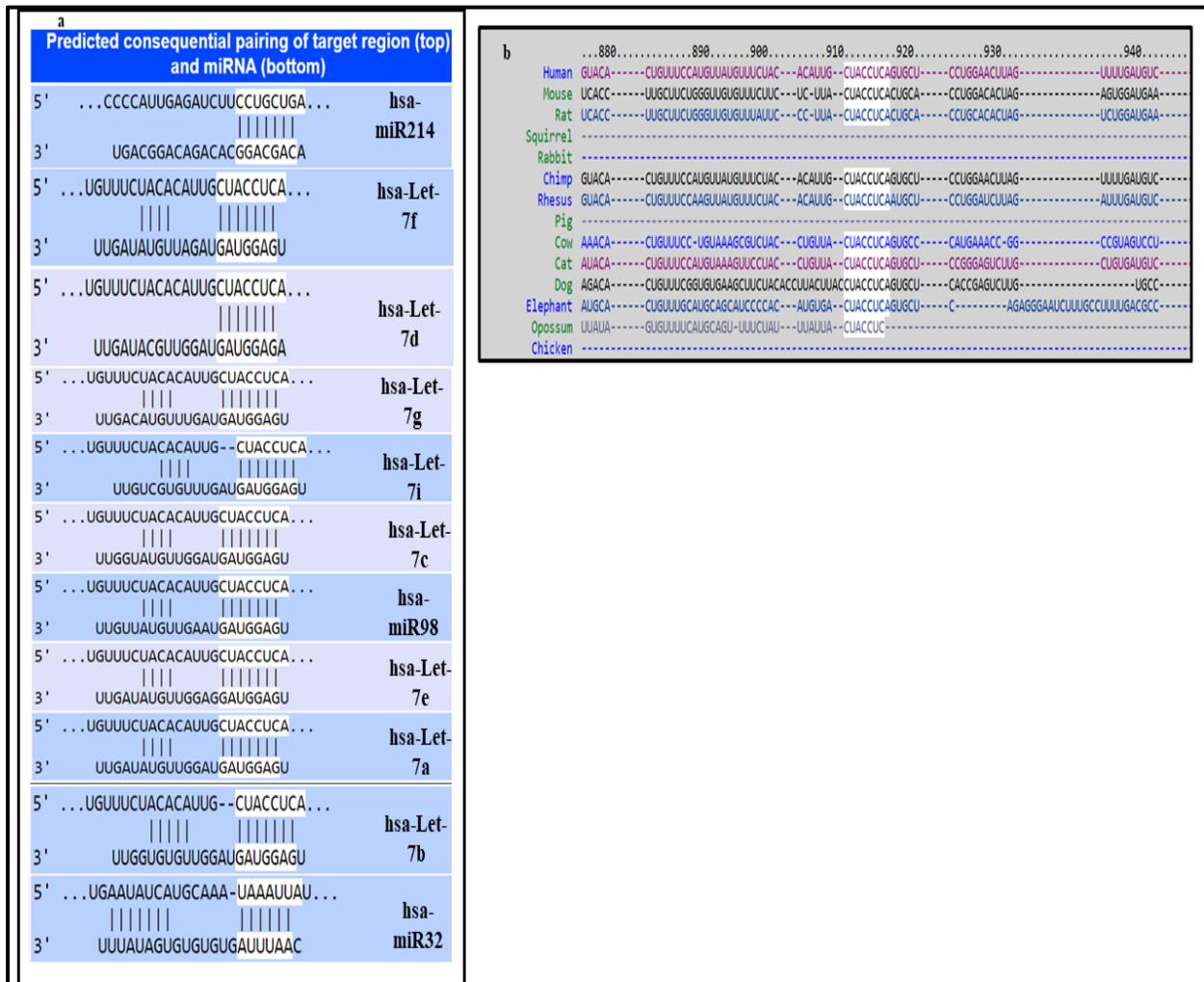
Supplementary Table 4 Target accessibility and probability profile for human Tmprss2 (3'UTR) – anti-siRNA binding

S.No	miRNA	sense siRNA	antisense siRNA	siRNA GC content	differential stability of siRNA duplex ends (kcal mol ⁻¹)	average unpaired probability for target site nucleotides	binding site disruption energy (kcal mol ⁻¹)
1	hsa-miR-214-3p	CUGGAUGACUUGAGAUGAATT	UUCAUCUCAAGUCAUCCAGTT	42.1	3.9	0.598	5.4
2	hsa-let-7a-5p	CACCAUGUUGGCCUCUUCATT	UGAAGAGGCCAACAUGGUGTT	52.6	2.7	0.604	16.1
3	hsa-let-7b-5p	CUGGAUGACUUGAGAUGAATT	UUCAUCUCAAGUCAUCCAGTT	42.1	3.9	0.604	5.4
4	hsa-let-7c-5p	CUGGAUGACUUGAGAUGAATT	UUCAUCUCAAGUCAUCCAGTT	42.1	3.9	0.605	5.4
5	hsa-let-7d-5p	GCUCCUAAUAAAGACAUAUACTT	GUAUGUCUUUAUUAGGAGCTT	36.8%	4.5	0.615	8.7
6	hsa-let-7e-5p	CACCAUGUUGGCCUCUUCATT	UGAAGAGGCCAACAUGGUGTT	52.6	2.7	0.605	16.0
7	hsa-let-7f-5p	CACCAUGUUGGCCUCUUCATT	UGAAGAGGCCAACAUGGUGTT	52.6	2.7	0.603	16.1
8	hsa-let-7g-5p	CACCAUGUUGGCCUCUUCATT	UGAAGAGGCCAACAUGGUGTT	52.6	2.7	0.596	16.2
9	hsa-let-7i-5p	CUGGAUGACUUGAGAUGAATT	UUCAUCUCAAGUCAUCCAGTT	42.1	3.9	0.600	5.3

Supplementary Figures



Supplementary Fig. 1 Binding interactions between miRNA and Tmprss2 enlisted by miRanda miRNA prediction tool. miRNA (top) and Tmprss2 (bottom).



Supplementary Fig. 2 a. Binding interactions between miRNA and Tmprss2 enlisted by TargetScan miRNA prediction tool. miRNA (top) and Tmprss2 (bottom) **b.** The sequence conservation for Tmprss2 across different species.

References

- Forero, D. A., González-Giraldo, Y., Castro-Vega, L. J., & Barreto, G. E. (2019). qPCR-based methods for expression analysis of miRNAs. *BioTechniques*, 67(4), 192-199.
- Kaur, T., John, A. A., Sharma, C., Vashisht, N. K., Singh, D., Kapila, R., & Kapila, S. (2020). miR300 intervenes Smad3/ β -catenin/RunX2 crosstalk for therapy with an alternate function as indicative biomarker in osteoporosis. *Bone*, 115603.